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Prevalence of Antibodies to *Toxoplasma gondii* in Raccoons (*Procyon lotor*) From an Urban Area of Northern Virginia

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ABSTRACT: Raccoons (*Procyon lotor*) are intermediate hosts for *Toxoplasma gondii*, and clinical toxoplasmosis in raccoons has been reported. A 2-yr serological survey was conducted to determine the prevalence of antibodies to *T. gondii* in raccoons collected from Fairfax County, Virginia, a suburban/urban area outside Washington, D.C. Serum samples from 256 raccoons were examined for *T. gondii* antibodies at a 1:50 dilution using the modified direct agglutination test. Results indicated that 216 (84.4%) of the raccoons had been exposed to *T. gondii*. Our results indicate that raccoons in this area of Virginia are frequently exposed to *T. gondii*. Domestic cats were common in the study area and may have served as a source of oocysts for raccoons and the food items of raccoons.

Raccoons (*Procyon lotor*) can serve as environmental monitors for several zoonotic diseases of humans (Bigler et al., 1975). Because they are omnivores, raccoons can acquire *Toxoplasma gondii* infections by ingesting oocysts from the environment or tissue cysts within infected prey or scavenged food sources. Clinical toxoplasmosis has been observed in naturally infected raccoons (Maurer and Nielsen, 1981). Raccoons with concurrent canine distemper virus infection are more likely to have toxoplasmosis than are those without this immunosuppressive virus (Moller and Nielsen, 1964; Maurer and Nielsen, 1981; Dubey et al., 1992). The present study was conducted to determine the serological prevalence of antibodies to *T. gondii* in raccoons from an urban area of northern Virginia.

The raccoons used in the present study originated in various locations of Fairfax County (38°42′N, 77°10′W), Virginia, a suburban/urban area outside Washington, D.C. The animals were live-trapped as part of a larger study of rabies in Fairfax County. Blood samples were collected from all trapped raccoons. The animals were released immediately after sampling was completed. The serum was collected, placed in a tube, and frozen at −70 C. Frozen sera were sent to the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, for agglutination testing.

The modified direct agglutination test (MAT) (Dubey and Desmonts, 1987) was used to examine sera for agglutinating antibodies to *T. gondii*. The MAT has been validated in raccoons experimentally infected with *T. gondii* (Dubey et al., 1993). A dilution of 1:50 was used to screen sera in the present study. We examined serum samples from 136 raccoons in the year 2000 and from another 120 in the year 2001. Positive raccoon sera from the 2001 sampling period were examined further at dilutions of 1:500 and 1:1,000.

Positive MAT test results were found in 110 (80.9%) of 136 raccoons from the year 2000 and in 106 (88.3%) of 120 raccoons from the year 2001. Total prevalence over the 2-yr study period was 216 of 256 (84.4%). Thirty-six (34.0%) of 106 positive samples from 2001 were MAT positive at a 1:500 dilution, and 31 (29.2%) of these 106 samples also were positive at a 1:1,000 dilution.

Our overall prevalence is higher than that reported by others for raccoons from Virginia and other regions of the United States (Table I). This may result from the higher sensitivity and specificity of the MAT compared to those of other serological tests used to determine the prevalence of *T. gondii*. Dubey et al. (1993) evaluated the performance of the MAT, Sabin-Feldman dye test (DT), latex agglutination test (LAT), and indirect hemagglutination test (IHT) using sera from experimentally infected raccoons. They found that the MAT detected antibodies earlier and at higher titers compared with the other serological tests (Dubey et al., 1993). Results of the DT and MAT were similar for identifying positive raccoons, whereas the LAT and IHT gave consistently fewer positive titers (Dubey et al., 1993).

Raccoons are susceptible to both *T. gondii* tissue cysts and oocysts, but clinical toxoplasmosis has not been reported in experimentally infected raccoons (Miller et al., 1972; Dubey et al., 1993). Tissue cysts of experimentally infected raccoons are more prevalent in the muscles than in the brain (Dubey et al., 1993). This is interesting, because clinical toxoplasmosis in raccoons is associated with encephalitis and immunosuppression (Moller and Nielsen, 1964; Maurer and Nielsen, 1981; Dubey et al., 1992).

We did not catch bobcats (*Lynx rufus*) during our trapping of raccoons, but we frequently caught domestic cats. We estimate that we trapped 10–15 cats for every 100 raccoons. Domestic cats are a probable source of *T. gondii* oocysts for raccoons and prey items of raccoons.

TABLE I. Prevalence of antibodies to *Toxoplasma gondii* in raccoons.

Location	No. examined/ No. positive (%)	Test*	Reference
California	25/12 (48)	IHAT	Franti et al. (1976)
California	7/2 (29)	IHAT	Riemann et al. (1975)
Florida	530/95 (18)	IHAT	Burridge et al. (1979)
Florida	24/7 (29)	MAT	Lindsay et al. (2001)
Georgia	67/22 (33)	DT	Walton and Walls (1964)
Illinois	379/184 (49)	MAT	Mitchell et al. (1999)
Iowa	885/134 (15)	MAT	Hill et al. (1998)
Iowa	30/20 (67)	MAT	Dubey et al. (1992)
Iowa	14/4 (29)	MAT	Smith et al. (1992)
Maryland	77/18 (24)	DT	Jacobs and Stanley (1962)
Massachusetts	25/23 (92)	MAT	Lindsay et al. (2001)
New Jersey	45/33 (73)	MAT	Dubey et al. (1992)
New Jersey	25/10 (40)	MAT	Lindsay et al. (2001)
Ohio	119/67 (56)	MAT	Dubey et al. (1992)
Pennsylvania	93/45 (48)	MAT	Dubey et al. (1992)
Pennsylvania	25/6 (24)	MAT	Lindsay et al. (2001)
South Carolina	72/25 (35)	MAT	Dubey et al. (1992)
Virginia	68/25 (37)	MAT	Dubey et al. (1992)
Virginia	256/216 (84)	MAT	Present study

* DT, Sabin-Feldman dye test; IHAT, indirect hemagglutination test; MAT, modified direct agglutination test.

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Toxoplasmosis in a Hawaiian Monk Seal (*Monachus schauinslandi*)

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ABSTRACT: *Toxoplasma gondii* infection in marine mammals is intriguing and indicative of contamination of the ocean environment with oocysts. *T. gondii* was identified in a Hawaiian monk seal (*Monachus schauinslandi*) that had visceral and cerebral lesions. Tachyzoites were found in the lymph nodes, spleen, diaphragm, heart, adrenal glands, and brain. A few tissue cysts were found in sections of the cerebrum. The diagnosis was confirmed serologically, by immunohistochemical staining with *T. gondii*-specific polyclonal rabbit serum, and by the detection of *T. gondii* DNA. The genotype was determined to be type III by restriction fragment length polymorphisms of the SAG2 gene. This is the first report of *T. gondii* infection in a Hawaiian monk seal.

Toxoplasma gondii infections have been reported in many homeothermic animals, including several species of marine mammals (Dubey and Beattie, 1988; Resendes et al., 2002; Dubey et al., 2003). Recently, concerns have been raised that *T. gondii* may be a cause of mortality in sea otters, which are an endangered species in U.S. waters (Thomas and Cole, 1996; Lindsay et al., 2001; Miller, Gardner, Kreuder et al., 2002). Viable *T. gondii* was isolated from 15 of 67 (Cole et al., 2000) and 24 of 75 (Miller, Gardner, Packham et al., 2002) dead sea otters, indicating that *T. gondii* infection is common in this animal. It has been suggested that sea otters become infected with *T. gondii* oocysts in the sea from freshwater coastal runoff (Miller, Gardner, Kreuder et al., 2002). The Hawaiian monk seal (*Monachus schauinslandi*) is an endangered pinniped in U.S. waters and its population has declined since 1950. We report fatal *T. gondii* infection in this host for the first time.

An adult male Hawaiian monk seal (RK07) was found dead in the surf zone on 22 January 2004 at Otsuka beach on the east side of the island of Kauai, near the town of Kapaa. The animal was covered with ice, and a necropsy examination was performed 15 hr later. This animal was in good nutritional condition as evidenced by normal blubber thickness. The submandibular, prescapular, and tracheobronchial lymph nodes were enlarged and darker than normal. The cranial lung lobes were edematous, glistening, and gelatinous bilaterally. The stomach contained 2 L of fluid, and the bile duct was enlarged. Helminths were found in the trachea and stomach.

Tissue specimens were fixed in 10% formalin and submitted to the Armed Forces Institute of Pathology, Washington, D.C., for histologic examination. Tissues were processed routinely, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Tissues were also forwarded to the Animal Parasitic Diseases Laboratory, Beltsville, Maryland, for *T. gondii* examination. Deparaffinized sections of tissues were stained immunohistochemically with *T. gondii* and *Neospora caninum* polyclonal sera as described (Lindsay and Dubey, 1989; Dubey et al., 2001). In addition, sections were allowed to react with anti-BAG1 polyclonal rabbit antibody specific for bradyzoites (McAllister et al., 1996). A sample of frozen blood was thawed, centrifuged, and the serum was tested for antibodies to *T. gondii* using the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987). For molecular confirmation, DNA was extracted from the lymph nodes of the monk seal using a DNAeasy tissue kit (Qiagen, Valencia, California) according to the manufacturer's instructions. The DNA sample was tested for the amplification of the *T. gondii*-specific SAG2 fragments (Howe et al., 1997), along with positive and negative controls. The PCR products were electrophoresed in a 2% agarose gel with molecular weight standards. Genotype was determined by restriction fragment length polymorphisms (RFLP) of the SAG2 gene (Howe et al., 1997).

Prominent lesions were observed in multiple tissues (lymph nodes, spleen, adrenal glands, diaphragm, and brain), and characterized by necrosis with variable numbers of extracellular and intracellular protozoal tachyzoites. The most severe lesions were within the lymph nodes (Fig.

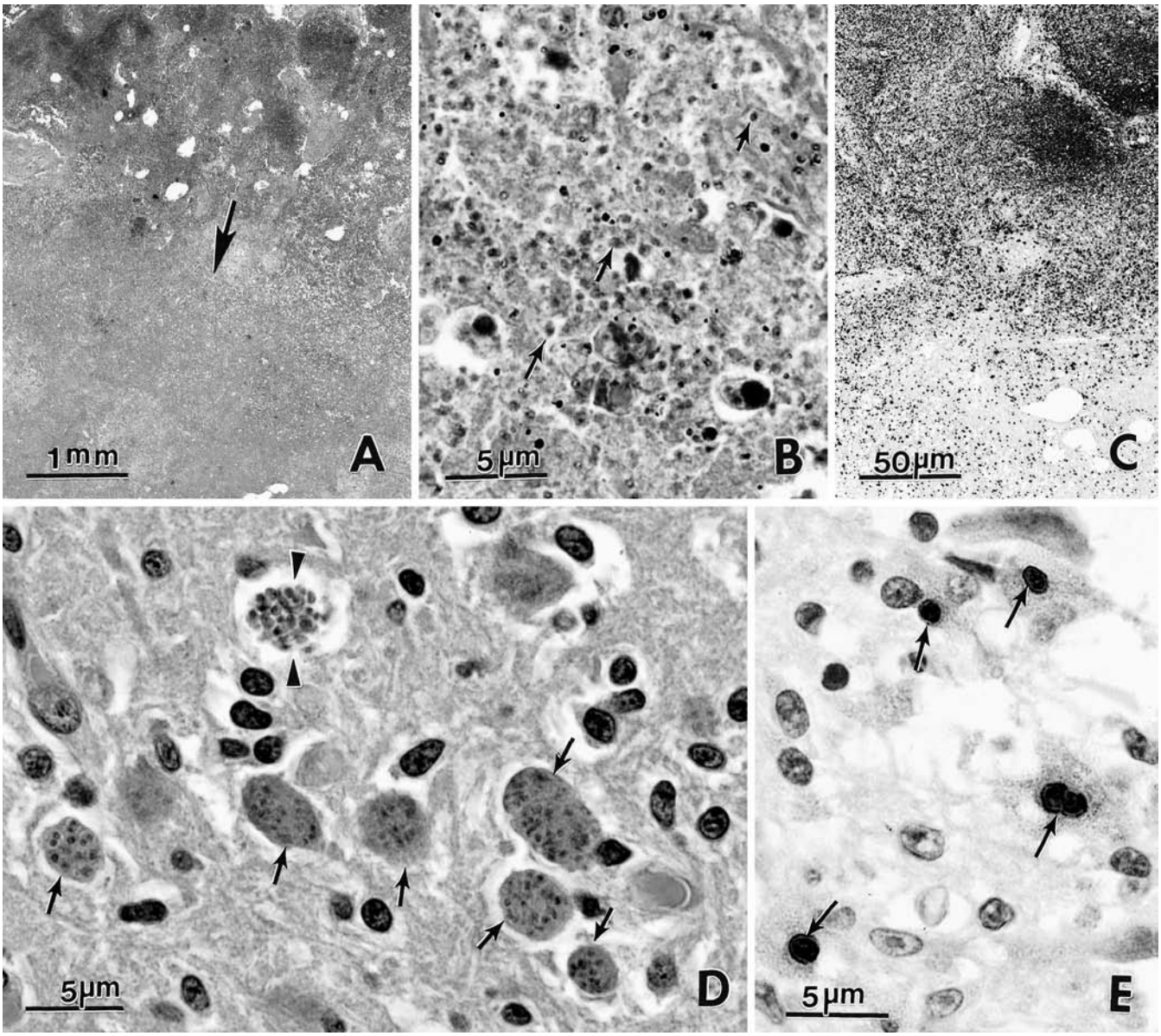

FIGURE 1. Lesions and *T. gondii* in sections of lymph nodes **(A–C)** and cerebrum **(D, E)** of the Hawaiian monk seal stained with hematoxylin and eosin **(A, B, D)** or with *T. gondii* antibodies **(C, E)**. **(A)** Note a large central area of necrosis (arrow). **(B)** Numerous extracellular tachyzoites (arrows). **(C)** Numerous *T. gondii* tachyzoites (all black areas). **(D)** Note 6 tissue cysts (arrows) and a group of tachyzoites (arrowheads). **(E)** Note that tachyzoites (arrows) stained immunohistochemically are larger in size than tachyzoites stained with hematoxylin and eosin in **(B)**.

1A–C). The medulla of the lymph nodes contained large areas of lytic necrosis, hemorrhage, and numerous extracellular protozoal tachyzoites. Scattered throughout the cortex of the lymph nodes were numerous intracellular tachyzoites. The spleen was similarly affected with large areas of necrosis containing both extracellular and intracellular tachyzoites. Within the adrenal cortex there were multifocal, variably sized areas of lytic necrosis with hemorrhage and a few tachyzoites. Less affected areas of the adrenal cortex contained few intracellular tachyzoites. There were few random, multifocal areas of necrosis and rare intracellular tachyzoites within the diaphragm. The heart had a single aggregate of intracellular tachyzoites. The brain had few scattered glial nodules, a few extracellular and intracellular tachyzoites, and few small tissue cysts (Fig. 1C, D).

Other histologic findings included minimal multifocal lymphocytic myelitis with gliosis and hemorrhage, moderate diffuse pulmonary congestion with alveolar edema, minimal multifocal epicardial nodular granulomatous steatitis with edema, mild focal histiocytic and neutrophilic gastritis with intralesional metazoan parasite, moderate multifocal testicular interstitial hemorrhage with minimal subacute epididymitis, and multiple intraluminal cestode larvae within the small intestine. In addition, there was moderate autolysis in several tissues with significant postmortem bacterial overgrowth.

Protozoa in sections of lymph nodes, spleen, adrenal glands, lung, and the cerebrum reacted positively with *T. gondii* polyclonal antibodies (Fig. 1C, E) and not with *N. caninum* antibodies. Tissue cysts in brain reacted with BAG 1 antibodies. Antibodies to *T. gondii* were found in a 1:100 dilution of the serum even though the sample was hemolyzed. Amplifications of the specific SAG2 products were noticed from the DNA obtained from the lymph node of the monk seal as well as the positive control. The isolate was found to have the type III genotype on RFLP.

The character of lesions suggests that the seal acquired *T. gondii* infection recently, most likely through ingestion of oocysts. There was severe necrosis of lymph nodes with numerous tachyzoites. The cerebral

lesions were localized, acute in nature, with only small tissue cysts present. In animals fed oocysts, initial lesions occur in the intestines and mesenteric lymph nodes, and some hosts can die before infection is established in the brain (Dubey and Beattie, 1988). Neural lesions are formed 2–3 wk after infection and initially consist of small areas of focal necrosis. With the passage of time, glial nodules are formed and tachyzoites begin to disappear from neural lesions.

The diagnosis of toxoplasmosis was confirmed in this animal immunohistochemically, by detection of antibodies, and DNA specific for *T. gondii*. The genotype of the isolate was found to be type III. All 48 isolates of *T. gondii* from sea otters genotyped thus far were either type II or a new genotype (Cole et al., 2000; Miller et al., 2004). The present case is the first indication of the presence of type III genotype in a marine mammal. It is also the first amplification of *T. gondii* DNA directly from the marine host. Previous data have been based on *T. gondii* isolates obtained by bioassay in cell culture or laboratory animals.

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Trematodes Associated with Mangrove Habitat in Puerto Rican Salt Marshes

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ABSTRACT: *Batillaria minima* is a common snail in the coastal estuaries of Puerto Rico. This snail is host to a variety of trematodes, the most common being *Cercaria caribbea* XXXI, a microphallid species that uses crabs as second intermediate hosts. The prevalence of infection was higher (7.1%) near mangroves than on mudflats away from mangroves (1.4%). Similarly, there was a significant positive association between the proportion of a site covered with mangroves and the prevalence of the microphallid. The association between mangroves and higher trematode prevalence is most likely because birds use mangroves as perch sites and this results in local transmission to snails.

A visit to the Center for Disease Control website on travelers' health underscores how parasite transmission can vary greatly from place to place, depending on environmental factors and the distribution of infective stages. In the present study, we investigated whether habitat (mangrove vs. mudflat) affected the prevalence of trematodes in snails. Transmission of trematodes to snails should be highest in habitats that support many final hosts (Hoff, 1941; Cort et al., 1960; Robson and Williams, 1970; Sousa and Grosholz, 1991; Sapp and Esch, 1994; Smith, 2001; Kube et al., 2002; Skirnisson et al., 2004). For example, infections in some snail species are more prevalent near the littoral zone where final hosts are more abundant (Sapp and Esch, 1994; Esch et al., 1997). Recently, Skirnisson et al. (2004) related spatial heterogeneity in the prevalence of larval trematodes infecting *Hydrobia ventrosa* to both biotic and abiotic variables; they assumed that these factors influenced attractiveness of the habitats for definitive hosts, which ultimately produced the observed spatial heterogeneity in infections in first intermediate hosts. Smith (2001) found that bird droppings were highest near dead mangroves, where birds perched, compared to mudflats. In California, however, direct associations occur between birds and trematodes

in free-ranging *Cerithidea californica* snails (Hechinger and Lafferty, 2005). In turn, these perch areas had higher transmission of trematodes to caged *Cerithidea scalariformis*. However, the association between parasitism and habitat in free-ranging snails was more ambiguous, perhaps because movement of free-ranging snails blurred existing spatial patterns of parasite transmission between mangroves and mudflats.

The estuarine snail *Batillaria minima* is abundant in shallow coastal lagoons in Puerto Rico. We assessed the association between trematode prevalence in *B. minima* and the amount of mangrove habitat at a site. We expected that the habitat for birds provided by mangroves would lead to higher transmission of trematodes and this, in turn, would result in a higher prevalence of trematodes in snails collected near mangroves. The results were consistent with the prediction that snails from mangrove habitats had a higher prevalence of trematodes.

We sampled trematodes from snails at 2 estuaries (Las Salinas and Cabo Rojo) on the southern shore of the island of Puerto Rico. Collection took place during 22 and 23 February 2004 at Laguna de Las Salinas and Laguna de Cabo Rojo, respectively. Laguna de Cabo Rojo is located 55 km west of Laguna de Las Salinas and both lagoons have similar hydrogeological characteristics. The dominant plant around the stations at both lagoons was the red mangrove (*Rhizophora mangle*). The more halophilic white and black mangroves (*Laguncularia racemosa* and *Avicenia germinans*) were present, but less abundant. The button mangrove (*Conocarpus erectus*) and the ''tachuelo'' shrub (*Pictetia aculeata*) occured at Laguna de Las Salinas. Birds were nearly absent during our visit (primarily due to the season) and we were not able to directly establish associations between bird abundance and trematode abundance.

Seven sites were chosen for sampling at Las Salinas. All were at least 50 m apart. Two of the estuary sites were adjacent to large stands of mangroves. The rest were on exposed ''flats'' in the estuary (nearby mangroves were either sparsely distributed or only present as a narrow fringe bordering the lagoon). Nine sites were chosen at Cabo Rojo (a more pristine area). Again, all were at least 50 m apart. Two sites were adjacent to large natural stands of mangroves. One site was located at a mangrove nursery that had many PVC stakes and young mangroves. The remaining 6 sites were mudflats.

Site descriptions were further refined by characterizing the amount of vegetation from Ikonos satellite photos of the 2 estuaries (taken in 2001). We used Arc GIS to establish 25-m radius buffers around the center of each sampling station, and then digitized the vegetation within those zones. Based on field observations, the vast majority of the vegetation was mangrove with minor portions consisting of shrubs and other small trees. Two sites at Cabo Rojo had changed substantially since the 2001 image. At one, many of the mangroves had died. At the other (nearby), a mangrove restoration project was underway. We reclassified the vegetation at these sites based on direct visual estimates.

In general, 100 *Batillaria minima* snails were collected within 10 m of the center of each site. We intentionally targeted larger snails (>12 mm) as estuarine snails are more likely to be exposed to parasites (Kuris, 1990). Snails were transported to the lab in nylon mesh bags and kept moist, until processing, with a daily short emersion in fresh seawater.

Each snail was measured by digital caliper to the nearest tenth of a mm. The shell of the snail was then cracked with a hammer, exposing the snail body. This was examined under a dissecting microscope for trematodes and the presence of gonad tissue. Infections were apparent as cercariae swimming in the dish and/or as sporocyst and redial stages in the visceral mass (typically, intramolluscan stages occurred where gonad tissue would normally be, adjacent to the digestive gland in the posterior shell spire). Trematodes were examined using a compound microscope and identified according to Cable (1956).

The data were analyzed to address what factors explained significant amounts of variation in parasitism among sites. Parasitism, the dependent variable, was expressed as a nominal variable (infected or uninfected) and analyzed with a logistic general linear model using JMP 5.0.1 software (by SAS, Cary, North Carolina). The following independent variables were used in the model to determine whether any had an independent association with parasitism: estuary (Las Salinas or Cabo Rojo), habitat (flat vs mangrove), and snail size. The complete model was run initially and inspected for significant effects and interactions among the independent variables.

After analyzing the data, it became apparent that vegetation was an important factor affecting parasites. We then used the percent cover of mangroves in the 25-m radius around the collection site as a continuous variable to replace the categorical habitat variable previously used. In addition to a logistic model using individual snails as replicates, we conducted a simple linear correlation analysis using sites as replicates.

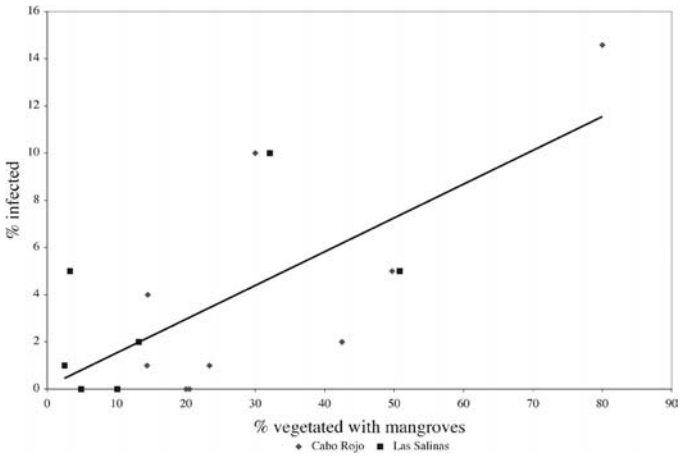

FIGURE 1. Association between trematode prevalence in the snail *Batillaria minima* (vertical axis) and mangrove cover (horizontal axis) in 2 Puerto Rican estuaries ($R^2 = 0.48$, $P = 0.003$).

From Las Salinas, 700 *B. minima* were dissected for parasites and 3.2% were infected. The average size of the snails was 14.4 ± 1.4 mm (±SD) and 45% of the uninfected snails showed evidence of gonad development. From Cabo Rojo, 815 *B. minima* were dissected for parasites and 3.7% were infected. The average size of the snails was 14.1 ±1.1 mm and 52% of the uninfected snails showed evidence of gonad development.

Three species of trematodes were found in *B. minima*. Only 1 species of trematode was found in each infected snail. The most common (96% of infected snails) was *Cercaria caribbea* XXXI Cable (1956) (a microphallid species that we experimentally determined uses crabs as second intermediate hosts (R. Hechinger, unpubl. obs.), and probably uses birds as final hosts). A xiphidiocercaria described by Cable (1956) as *Cercaria caribbea* XXXIII (probably a renicolid species that uses mollusks or polychaetes as second intermediate host and birds as final hosts) was found from a single snail (*Cercaria caribbea* XXXIII was more common on the outer shore of the estuary; data not shown). A third cercaria, an undescribed oculoleptocercogymnocephalus distome cercaria, was found in a single snail from Cabo Rojo. The taxonomic affinities of this species are unknown, but it may use fish as final hosts.

The statistical analysis of infections in *B. minima* showed no significant interactions among independent variables. Estuary was not significantly associated with parasitism and was dropped from the final model. In the final model, snail size (1 df, Chi square = 8.5, $P = 0.0035$) and habitat type (1 df, Chi square = 24.2, $P \leq 0.0001$) were significantly associated with parasitism. Snails accumulate infections over time and, therefore, larger snails were more likely to be infected than were small snails. Snails from sites near mangroves had a higher prevalence of infection (7.1%) than did snails from mudflats (1.4%).

Using percent vegetation as a variable in place of habitat gave similar results. Estuary was not significant (df 1, Chi square = 0.55, $P = 0.46$), and vegetation was positively associated with parasitism (df 1, Chi square = 23.13, $P < 0.0001$). There was no significant interaction between estuary and percent vegetation, indicating that the relationship between vegetation and parasitism was the same in each estuary. The latter analysis uses snails as replicates but the most appropriate unit of replication is arguably the site. For this reason, we also addressed the association between vegetation and parasitism by correlating the prevalence of parasitism at a site against the percent vegetation at a site. A positive association resulted (Fig. 1, 16 sites, $R^2 = 0.48$, $P = 0.003$).

The distribution of trematodes in snails at 2 Puerto Rican estuaries is associated with the distribution of mangroves. This result has a logical interpretation. The most common invertebrate (other than snails) visible on these flats is the fiddler crab *Uca rapax rapax*. Second intermediate

hosts, such as fiddler crabs, are infected by trematode stages that swim from snails. Birds become infected with trematode adults when they forage on prey, like fiddler crabs, that are infected with trematode metacercariae. After the trematodes mature in the birds, they pass their eggs with the birds' excreta and these eggs may then infect nearby snails. The distribution of birds' feces and, therefore, infections to snails, is probably higher in roosting areas such as mangroves. The highest prevalence of trematodes occurred at the mangrove nursery site, suggesting that this area provided roosting or foraging habitats for birds. This is consistent with the study by Smith (2001), which found direct associations between bird abundance at dead mangrove perches and trematode prevalence in caged snails. However, Smith (2001) found no such association between birds and parasitism in free-ranging snails. In contrast, we found that snail movement did not fully obscure associations between habitat and parasitism. This could occur if *B. minima* moves less or lives shorter than *C. scalariformis* or could be a result of larger distances between sampling sites (our sites were >50 m apart while Smith's were >10 m apart).

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Effects of High Pressure Processing on Infectivity of *Toxoplasma gondii* Oocysts for Mice

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ABSTRACT: High pressure processing (HPP) has been shown to be an effective non-thermal method of eliminating non-spore forming bacteria from a variety of food products. The shelf-life of the products is extended and the sensory features of the food are not or only minimally effected by HPP. The present study examined the effects of HPP using a commercial scale unit on the viability of *Toxoplasma gondii* oocysts. Oocysts were exposed from 100 to 550 MPa for 1 min in the HPP unit and then HPP treated oocysts were orally fed to groups of mice. Oocysts treated with 550 MPa or less did not develop structural alterations when viewed with light microscopy. Oocysts treated with 550 MPa, 480 MPa, 400 Mpa, or 340 MPa were rendered noninfectious for mice. Mice fed oocysts treated with no or 100 to 270 MPa became infected and most developed acute toxoplasmosis and were killed or died 7 to 10 days after infection. These results suggest that HPP technology may be useful in the removal of *T. gondii* oocysts from food products.

Toxoplasma gondii is an important parasite of humans and other warm-blooded animals. There are about 1,500,000 cases of toxoplasmosis in the United States each yr and about 15% of those infected have clinical signs (Mead et al., 1999; Jones, Kruszon-Moran et al., 2001). Congenital toxoplasmosis has long been recognized because of the devastating results it can have on the infected fetus (Jones, Lopez, et al., 2001). Toxoplasmosis is also a frequent and fatal complication in patients with AIDS or those that receive organ transplantation (Soave, 2001). The annual economic impact of toxoplasmosis in the human population in the United States is about $7.7 billion (Buzby and Roberts, 1996). It is not yet possible to determine if tissue cysts in meat or oocysts from cats are the main source of human infection in the United States.

High pressure processing (HPP) has been shown to be an effective non-thermal means of eliminating non-spore forming bacteria from a variety of food products (see Tewari et al., 1999). The shelf-life of the products is extended and the sensory features of the food are not or only minimally effected by HPP. Other advantages of HPP over tradi-

tional thermal processing include reduced processing times; minimal heat damage; retention of freshness, flavor, texture, and color; no vitamin C loss; no undesirable changes in food during pressure-shift freezing due to reduced crystal size and multiple ice-phase forms; and minimal undesirable functionality alterations (see Tewari et al., 1999).

Little has been done with parasites and HPP. Ohnishi et al. (1992, 1994) determined that pressures of greater than 200 MPa (1 MPa = 145 psi = 10 bar = 9.87 atm) killed 8-wk-old *Trichinella spiralis* larvae. Gamble et al. (1998) determined 55 to 60 MPa did not kill all *T. spiralis* larvae in pork tenderloin or diaphragm. Treatment at 200 MPa for 10 min at temperatures between 0 and 15 C kills *Anisakis simplex* larvae with a lack of motility being used as an indicator of larval death (Molina-Garcia and Sanz, 2002). Dong et al. (2003) found HPP effective in killing *A. simplex* in salmon but that the pressures and exposure times needed to reach 100% killing caused a significant change in the color of treated salmon fillets. Slifko et al. (2000) examined the effects of 550 MPa on *Cryptosporidium parvum* oocysts in apple and orange juice. They determined that a 1 min exposure at 550 MPa was 100% effective in decreasing infectivity of oocysts for cell cultures. The present study was done to determine the effects of HPP on oral infectivity of treated *T. gondii* oocysts for mice.

A *T. gondii* naïve cat was fed tissues from a naturally infected chicken from New England containing tissue cysts of *T. gondii* (Dubey et al., 2003). The cat was housed and infected in a cat colony at the United States Department of Agriculture, Animal Parasite Diseases Laboratory, Beltsville, Maryland (Dubey, 1995). Feces containing unsporulated oocysts were collected, sporulated and purified using Sheather's sugar solution, and the suspension of purified oocysts sent on cool packs to the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia. Oocysts were counted in a hemocytometer and dose of oocysts administered to mice (Table I) were based on visual counts.

Groups of 2 each, female, CD-1 mice were used to determine the effects of high hydrostatic pressure treatment on the infectivity of *T. gondii* oocysts. All mice were orally fed control or HHP treated oocysts using animal feeding needles. Impression smears were made from the mesentric lymph nodes or lungs of mice that died and were examined unstained by light microscopy for tachyzoites. Five to 8 wk post-inoculation (PI), all surviving mice were bled from the retro-orbital plexus, serum collected, and assayed for antibodies to *T. gondii* in a modified direct agglutination assay (Dubey and Desmonts, 1987). The brains from all mice were examined for *T. gondii* tissue cysts by squash preparations. Oocysts exposed to various HPP treatments were examined using an Olympus BX60 microscope equipped with differential contrast optics.

Oocysts were placed in Hanks' balanced salt solution (HBSS) (Experiment 1), or distilled water (Experiments 2–4) in sealable leak proof bags. The bags were manually compressed to force out air and then sealed with a sealing machine. The bags were placed in additional bags and vacuum-sealed. The vacuum-sealed bags were used for HPP. All studies were done in a Quintus food press QFP 35L–600 (Flow International Corporation, Kent, Washington). Mice were each fed 5×10^4 to 4×10^6 HPP treated oocysts (Table I). Control samples were handled in the same manner but placed in the treatment tank of the HPP unit and not exposed to HPP treatment.

No alterations were seen in the structure of sporozoites or sporocysts in oocysts exposed to any of the HPP treatments in any of the experiments. The walls of oocysts exposed to HPP treatment also appeared normal.

All control mice in all experiments developed acute toxoplasmosis and all died or were killed when ill except for 1 mouse in experiment 1. This mouse was *T. gondii* brain tissue cyst positive when examined 8 wk PI. Pressures of 340 MPa or above were effective in rendering oocysts nonviable. Treatment of oocysts using HPP at 100 MPa, 140 MPa, 200 Mpa, or 270 MPa for 1 min did not cause a reduction in infectivity for mice (Table I).

Pressures of 550 MPa are routinely reached by commercial HPP machines. The current study indicates that HPP at 340 MPa for 1 min can be used to inactivate *T. gondii* oocysts. As mentioned previously, little work has been done on HPP and parasite inactivation. The results of our study are similar to those reported by Slifko et al. (2000) for *C. parvum* oocysts in apple and orange juice and 550 MPa for 1 min.

TABLE I. Experimental protocol and results of high-pressure processing on the infectivity of sporulated *Toxoplasma gondii* oocysts for mice.

Pressure (in MPa)	Exposure time*	No. oocysts fed/mouse	No. fed/No. infected†
Experiment 1			
550	60	5×10^4	2/0
480	60	5×10^4	2/0
400	60	5×10^4	2/0
340	180	5×10^4	2/0
340	120	5×10^4	2/0
340	90	5×10^4	2/0
340	60	5×10^4	2/0
0	0	5×10^4	2/2
Experiment 2			
550	60	5×10^5	2/0
480	60	5×10^5	2/0
400	60	5×10^5	2/0
340	60	5×10^5	2/0
0	0	5×10^5	2/2
Experiment 3			
270	60	5×10^4	2/2
200	60	5×10^4	2/2
140	60	5×10^4	2/2
100	60	5×10^4	2/2
0	0	5×10^4	2/2
Experiment 4			
550	60	4×10^6	2/0
550	60	2×10^6	2/0
550	60	1×10^6	2/0
550	60	5×10^5	2/0
0	0	5×10^5	2/2

* Exposure time in sec.
† Number of mice fed oocysts/number of mice positive for *T. gondii.*

High pressure is able to inactivate or destroy prokaryotic and eukaryotic cells because it affects biochemical molecules required for metabolism. Timson and Short (1965) reported that proteins are denatured under hydrostatic pressures. Pressure induces a decrease of protein volume as well as denaturation. As widely recognized, conformational changes of protein by pressure strongly depend on the volume effect caused by hydration (Ishizaki et al., 1995). Pressure has a volume decreasing effect and disrupts hydrophobic interactions (Balny and Masson, 1993). High pressure can result in the denaturation of proteins and their gelatinization. High pressure denaturation of several proteins, ovalbumin, bovine serum albumin, and beta-lactoglobulin, was assessed by spectrofluorometry, specific rotation analysis, and differential scanning calorimetry and compared with heat and chemical denaturation. In all cases, the denaturation caused by high pressure was similar to that caused by the cleavage of hydrogen bonds with urea or guanidine hydrochloride. The studies showed that hydrogen bonds holding together alpha-helical structures broke down, resulting in an unfolding of the protein chains (Hayakawa et al., 1996). Recent research has demonstrated that pressure levels above 100–200 MPa often induce on proteins the dissociation of oligomeric structure into their subunits, partial unfolding and denaturation of monomeric structures, protein aggregation, and protein gelation (Mozhaev et al., 1996; Heremans et al., 1997). The pressure from which these modifications appear, depend on the treated samples, temperature, and protein concentration. Research on the effects of high pressure on enzyme activities have clearly demonstrated that high pressure can affect the three dimensional structure of enzyme molecules leading to their irreversible inactivation. Numerous data have been elaborated on the kinetic inactivation parameters and kinetic model fitting pressurized isolated enzymes (Lemos et al., 1999).

Biomembranes have also been identified as a site affected by pressure

(Hoover et al., 1989). Biological membranes are composed by a bilayer of phospholipids with embedded functional proteins that, among others, play an important role in transporting ions and other substances across the membrane. It has been observed that lipid bilayers undergo phase transitions under pressure. Physiological phase corresponds to a liquid-crystalline phase, in which the hydrocarbon chains of the lipid bilayers are conformationally disordered (San Martin et al., 2002).

Coccidial oocysts have been detected in shellfish (see Fayer et al., 2004) and a variety of fresh produce (Ho et al., 2000; Dillingham et al., 2002; Doller et al., 2002). Studies on the efficacy of HPP on inactivating coccidial oocysts in fresh shellfish and on produce are logical avenues of research in preventing human food borne infections with coccidial parasites.

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